(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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22 February 2001 (22.02.2001)

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WO 01/12803 A3

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(30) Priority Data:
60/149,313 17 August 1999 (17.08.1999) US

(71) Applicant *(for all designated States except US)*: **BETH ISRAEL DEACONESS MEDICAL CENTER, INC.** [US/US]; 1 Deaconess Road, Boston, MA 02215 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **INOUYE, Roger, T.** [US/US]; 23 Roberts Road, Wellesley, MA 02481 (US). **TORRES-VIERA, Carlos** [VE/VE]; Calle Andrea de Ledesma, Qta La Torrera, Urb Sorocaima, Caracas, Venezuela (VE). **MOELLERING, Robert** [US/US]; 49 Longfellow Road, Wellesley Hills, MA 02481-5220 (US). **GOLD, Howard** [US/US]; Apartment 610, 135 Pleasant Street, Brookline, MA 02446-3489 (US). **ELIOPOULOS, George, M.** [US/US]; 5 Laurel Circle, Needham, MA 02494 (US).

(74) Agent: **PLUMER, Elizabeth, R.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

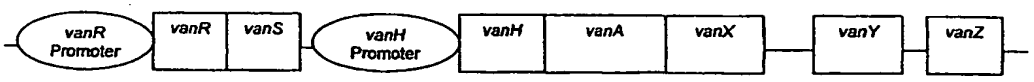
Published:
— *with international search report*

(88) Date of publication of the international search report:
18 October 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12803 A3

(54) Title: METHODS AND COMPOSITIONS FOR RESTORING ANTIBIOTIC SUSCEPTIBILITY IN GLYCOPEPTIDE-RESISTANT *ENTEROCOCCUS*



(57) Abstract: Methods and compositions for reducing vancomycin resistance in a vancomycin resistant organism is provided. The methods involve delivering to the organism an isolated nucleic acid molecule that hybridizes to a target vancomycin gene and/or that serves as a *VanR*-responsive promoter decoy.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 April 2001 (18.04.01)	
International application No. PCT/US00/22086	**Applicant's or agent's file reference** B0662/7036WO
International filing date (day/month/year) 11 August 2000 (11.08.00)	**Priority date** (day/month/year) 17 August 1999 (17.08.99)
Applicant INOUYE, Roger, T. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 February 2001 (20.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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(74) Agent: **PLUMER, Elizabeth, R.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

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Published:
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(88) Date of publication of the international search report:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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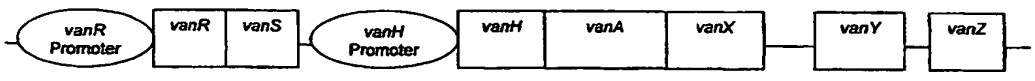

(57) Abstract: Methods and compositions for reducing vancomycin resistance in a vancomycin resistant organism is provided. The methods involve delivering to the organism an isolated nucleic acid molecule that hybridizes to a target vancomycin gene and/or that serves as a *VanR*-responsive promoter decoy.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/[redacted] 00/22086

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/315 C12N15/11 C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 92 07942 A (PASTEUR INSTITUT) 14 May 1992 (1992-05-14) the whole document, in particular pages 7, 46 and 51 ---	24-27,29 1-6,8, 10-17,19
Y	WO 90 00624 A (BAYLOR COLLEGE MEDICINE) 25 January 1990 (1990-01-25) the whole document, in particular page 4 line 7 to page 5 line 25 ---	1-17,19
A	PETER MITCHELL: "Facing up to antibiotic resistance" PHARMAPROJECTS MAGAZINE, vol. 3, no. 8, June 1998 (1998-06), pages 16-20, XP000943900 the whole document, in particular pages 18-19 --- -/--	1-23,28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2001	18.04.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Julia, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 12205 A (VIRUS RESEARCH INST INC ;BEATTIE DAVID T (US)) 26 March 1998 (1998-03-26) page 3 last paragraph to page 4 first paragraph	1-23,28
X	WO 96 08582 A (BERGERON MICHEL G ;OUELLETTE MARC (CA); ROY PAUL H (CA)) 21 March 1996 (1996-03-21) the whole document, in particular page 17, page 24 example 9, page 26 example 13 and Table 8	24-26
P,X	DATABASE GALE GROUP NEWSLETTER DB [Online] D.J. DENOON: "Gene-Based strategy reverses vancomycin resistance" XP002154962 Database accession number 56646980 abstract & Gene Therapy Weekly 1999, Oct 18	1-6, 10-23,28
Y	STEFAN EVERS AND PATRICE COURVALIN: "Regulation of VanB-type vancomycin resistance gene expression by the VanSB-VanRB two-component regulatory system in Enterococcus faecalis V583" JOURNAL OF BACTERIOLOGY, vol. 178, no. 5, March 1996 (1996-03), pages 1302-1309, XP002153486 US the whole document	1-5,7, 13-15
X	WO 94 14961 A (PASTEUR INSTITUT ;ARTHUR MICHEL (FR); DUTKA MALEN SYLVIE (FR); EVE) 7 July 1994 (1994-07-07)	24,25,27
Y	the whole document, in particular pages 6 and 8-10	1-5,7, 13-15
Y	F. NAVARRO AND P. COURVALIN: "Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in Enterococcus casseliflavus and Enterococcus flavescens" ANTIMICROB AGENTS CHEMOTHER, vol. 38, no. 8, August 1994 (1994-08), pages 1788-1793, XP000984075 the whole document	1-5,8, 13-15

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	B. CASADEWALL AND P. COURVALIN: "Characterization of the VanD glycopeptide resistance gene cluster from Enterococcus faecium BM4339" JOURNAL OF BACTERIOLOGY, vol. 181, no. 12, June 1999 (1999-06), pages 3644-3648, XP002153485 US the whole document ---	1-5,9, 13-15
X	WO 99 01571 A (MODRUSAN ZORA D ;ID BIOMEDICAL CORP (CA)) 14 January 1999 (1999-01-14) thw whole document, in particular claim 4 ---	24-27
X	M. ARTHUR ET AL., : "Regulated interactions between partner and non-partner sensors and response regulators that control glycopeptide resistance gene expression in enterococci" MICROBIOLOGY, vol. 145, no. PT8, August 1999 (1999-08), pages 1849-1858, XP000986365 the whole document, in particular paragraph bridging pages 1856-1857 and figure 2d ---	20,22
Y	GRISSOM-ARNOLD J ET AL: "INDUCTION OF VANA VANCOMYCIN RESISTANCE GENES IN ENTEROCOCCUS FAECALIS: USE OF A PROMOTER FUSION TO EVALUATE GLYCOPEPTIDE AND NONGLYCOPEPTIDE INDUCTION SIGNALS" MICROBIAL DRUG RESISTANCE,LIEBERT,US, vol. 3, no. 1, 1997, pages 53-64, XP000944092 ISSN: 1076-6294 the whole document, in particular page 61 rigth column ---	20,22
Y	MOELLERING R C: "ANTIBIOTIC RESISTANCE: LESSONS FOR THE FUTURE" CLINICAL INFECTIOUS DISEASES,THE UNIVERSITY OF CHICAGO PRESS, CHICAGO, IL,US, vol. 27, no. SUPP. 01, August 1998 (1998-08), pages S135-S140, XP000943873 ISSN: 1058-4838 the whole document, in particular page S138 rigth column last paragraph and page 139 rigth column --- -/--	20,22

4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARTHUR M ET AL: "THE VANS-VANR TWO-COMPONENT REGULATORY SYSTEM CONTROLS SYNTHESIS OF DEPSIPEPTIDE PEPTIDOGLYCAN PRECURSORS IN ENTEROCOCCUS FAECIUM BM4147" JOURNAL OF BACTERIOLOGY,WASHINGTON, DC,US, vol. 174, no. 8, April 1992 (1992-04), pages 2582-2591, XP000944110 ISSN: 0021-9193 cited in the application the whole document, in particular page 2587 left column and page 2588 left column second full-paragraph -----	20,22

INTERNATIONAL SEARCH REPORT

itional application No.
PCT/US 00/22086

Box I Observati ns where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-23 as far as they comprise in vivo (therapeutic) methods, are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 13-15, 24-27, 29 (partial) and 6, 10-12, 16-17, 19 (complete)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism at least one anti-sense vancomycin resistance molecule under conditions to inhibit expression of a vancomycin resistance gene, wherein said vancomycin resistant organism is a vanA resistant organism and the anti-sense molecule is selected from the group consisting of a vanA antisense molecule, a vanR antisense molecule, a vanS antisense molecule, a vanH antisense molecule, a vanX antisense molecule, a vanY antisense molecule and a vanZ antisense molecule. Said method wherein the anti-sense vancomycin resistance molecule hybridizes to the complete vanA gene sequence or to a conserved region (from 10 to 30 nucleotides) thereof (encodes an active site of the ligase) or to the complete vanX gene sequence or to a conserved region thereof. Said method wherein introducing the anti-sense vancomycin resistance molecule comprises contacting the vancomycin resistant organism with at least one vector (enterococcal shuttle vector, bacteriophage, peptide nucleic acid molecule, enterococcal conjugative transposon or a pheromone-responsive plasmid) comprising one or more vanA "anti-sense vancomycin resistance molecules" under conditions to allow the vector to enter the organism and inhibit expression of one or more vancomycin resistance genes.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanA resistance/VanA gene cluster of SEQ ID No.: 1 (which includes vanR, SEQ ID No.: 18; vanS, SEQ ID No.: 19; vanH, SEQ ID No.: 20; vanA, SEQ ID No.: 21; vanX, SEQ ID No.: 22; vanY, SEQ ID No.: 23; vanZ, SEQ ID No.: 24 and conserved sequences thereof) SEQ ID No.: 5-10. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

2. Claims: 1-5, 13-15, 24-27, 29 (partial) and 7 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanB resistant organism and the anti-sense molecule is selected from the group consisting of a vanRB antisense molecule, a vanSB antisense molecule, a vanYB antisense molecule, a vanW antisense molecule, a vanHB antisense molecule and a vanXB antisense molecule.

An isolated nucleic acid that hybridizes under stringent

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

conditions to a nucleic acid molecule selected from the VanB resistance/VanB gene cluster of SEQ ID No.: 2 (which includes vanRB, SEQ ID No.: 26; vanSB, SEQ ID No.: 27; vanYB, SEQ ID No.: 28; vanHB, SEQ ID No.: 29; vanB, SEQ ID No.: 30; vanXB, SEQ ID No.: 31; vanW, SEQ ID No.: 32 and conserved sequences thereof) SEQ ID No.: 11-12. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

3. Claims: 1-5, 13-15, 24-27, 29 (partial) and 8 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanC resistant organism and the anti-sense molecule is selected from the group consisting of a vanC antisense molecule or vanC-2.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanC resistance (SEQ ID No.: 3) mediated by vanC-2 gene (SEQ ID No.: 33). A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

4. Claims: 1-5, 13-15, 24-27, 29 (partial) and 9 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanD resistant organism and the anti-sense molecule is selected from the group consisting of a vanD antisense molecule, a vanRD antisense molecule, a vanSD antisense molecule, a vanYD antisense molecule, a vanHD antisense molecule and a vanXD antisense molecule.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanD resistance/VanD gene cluster of SEQ ID No.: 4 (which includes vanRD, SEQ ID No.: 34; vanSD, SEQ ID No.: 35; vanYD, SEQ ID No.: 36; vanHD, SEQ ID No.: 37; vanD, SEQ ID No.: 38; vanXD, SEQ ID No.: 39 and conserved sequences thereof) SEQ ID No.: 13. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

5. Claim : 20 and 22 (partial)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a vanH promoter in the organism, wherein the vanH promoter is not operatively coupled to a vancomycin resistance gene of the organism. Said method wherein the vanH promoter is contained on an enterococcus vector and enhancing expression comprises introducing into the organism

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism.

6. Claims: 18, 21, 23, 28 (complete) and 20, 22 (partial)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a vanH promoter in the organism to an amount sufficient to reduce vancomycin resistance in the organism, wherein the vanH promoter is operatively coupled to an antisense vancomycin resistance molecule (or if not operatively coupled then an antisense vancomycin resistance molecule operatively coupled to a vanH promoter is coadministered). Said method wherein the vanH promoter and the antisense vancomycin resistance molecule are contained on an enterococcus vector and enhancing expression comprises introducing into the organism an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism.

A method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism a vector comprising a VanR-responsive promoter (vanH) operatively coupled to the vanA antisense molecule. A vector comprising a vanH promoter operatively coupled to an isolated nucleic acid molecule that hybridizes under strinhgent conditions to a nucleic acid molecule selected from the group consisting of SEQ ID No.: 1-13. An isolated vancomycin resistant organism comprising such a vector.

INTERNATIONAL SEARCH REPORT

[redacted]on patent family members

Interna[redacted]al Application No
PC[redacted] 00/22086

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9207942 A	14-05-1992	FR 2668489 A	30-04-1992
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		EP 0996743 A	03-05-2000

4

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B0662/7036WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/22086	11/08/2000	17/08/1999
International Patent Classification (IPC) or national classification and IPC C12N15/11		
Applicant BETH ISRAEL DEACONESS MEDICAL CENTER, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 15 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/02/2001	11.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Julia, P Telephone No. +49 89 2399 8410

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/22086

I. Basis of th r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-31 as originally filed

Claims, No.:

1-29 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-19, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-6, 10-29 .

because:

☒ the said international application, or the said claims Nos. 1-6, 10-29 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 6, 10-12, 16-19, 21, 23, 28 (complete); 1-5, 13-15, 20, 22, 24-27, 29 (partial).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-5, 13-15, 20, 22 (partial); 6, 10-12, 16-17, 19, 20, 22 (complete)
	No:	Claims 24-27, 29 (partial)

Inventive step (IS)	Yes:	Claims	20, 22 (partial); 18, 21, 23, 28 (complete)
	No:	Claims	1-5, 13-15, 20, 22, 24-27, 29 (partial); 6, 10-12, 16-17, 19 (complete)
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-6, 10-29 (see citations and explanations)

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Additional r marks to item I :

A "Sequence Listing" has been filed with the present application. This "Sequence Listing" comprises SEQ ID No.: 1 to SEQ ID No.: 39 (pages 1-19).

2. Additional remarks to item II :

The priority documents pertaining to the present application were not available at the time of establishing this international preliminary examination report (IPER). Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (17.08.99). If it later turns out that this is not correct, the document Database Gale Group Newsletter DB, AN=56646980 & D.J. Denoon, Gene Therapy Weekly 1999, Oct. 18 cited in the International Search Report (ISR) could become relevant to assess whether the claimed subject matter of the present application satisfies the criteria set forth in Article 33 (1) PCT.

3. Additional remarks to item III :

i) upon invitation to pay for additional examination fees or to restrict the claimed subject matter (letter 03.07.01), the applicant with letter dated 27.07.01 has paid a further examination fee and requested the examination of the first and sixth group of inventions identified bellow under "Additional remarks to item IV", i.e. claims 1-5, 13-15, 24-27, 29 (partial) and claims 6, 10-12, 16-17, 19 (complete) (first group) and claims 20, 22 (partial) and claims 18, 21, 23, 28 (complete) (sixth group). Thus, the present IPER only concerns the subject matter of these claims.

ii) moreover, the attention of the Applicant is also drawn to the fact that the subject matter of examined claims 1-6 and 10-29 (complete and/or partial) can be seen as directed to methods for treatment of the human or animal body (insofar the claimed subject matter comprises methods in vivo too) and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT too (see below under "Additional remarks to item V").

4. Additional remarks to item IV :

The IPEA agrees with the non-unity objection originally raised by the International Search Agency (ISA) (Rule 13 PCT). The following group of inventions have been identified :

i) claims 1-5, 13-15, 24-27, 29 (partial) and 6, 10-12, 16-17, 19 (compl t): a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism at least one anti-sense vancomycin resistance molecule under conditions to inhibit expression of a vancomycin resistance gene, wherein said vancomycin resistant organism is a **vanA** resistant organism and the anti-sense molecule is selected from the group consisting of a vanA antisense molecule, a vanR antisense molecule, a vanS antisense molecule, a vanH antisense molecule, a vanX antisense molecule, a vanY antisense molecule and a vanZ antisense molecule. Said method wherein the anti-sense vancomycin resistance molecule hybridizes to the complete **vanA** gene sequence or to a conserved region (from 10 to 30 nucleotides) thereof (encodes an active site of the ligase) or to the complete vanX gene sequence or to a conserved region thereof. Said method wherein introducing the anti-sense vancomycin resistance molecule comprises contacting the vancomycin resistant organism with at least one vector (enterococcal shuttle vector, bacteriophage, peptide nucleic acid molecule, enterococcal conjugative transposon or a pheromone-responsive plasmid) comprising one or more **vanA** "anti-sense vancomycin resistance molecules" under conditions to allow the vector to enter the organism and inhibit expression of one or more vancomycin resistance genes. An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the **VanA** resistance /**VanA** gene cluster of SEQ ID No.: 1 (which includes vanR, SEQ ID No.: 18; vanS, SEQ ID No.: 19; vanH, SEQ ID No.: 20; vanA, SEQ ID No.: 21; vanX, SEQ ID No.: 22; vanY, SEQ ID No.: 23; vanZ, SEQ ID No.: 24 and conserved sequences thereof) SEQ ID No.: 5-10. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

ii) claims 1-5, 13-15, 24-27, 29 (partial) and 7 (complete) : the same method as invention group 1, but wherein said vancomycin resistant organism is a **vanB** resistant organism and the anti-sense molecule is selected from the group consisting of a vanRB antisense molecule, a vanSB antisense molecule, a vanYB antisense molecule, a vanW antisense molecule, a vanHB antisense molecule and a vanXB antisense molecule. An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the **VanB** resistance /**VanB** gene cluster of SEQ ID No.: 2 (which includes vanRB, SEQ ID No.: 26; vanSB, SEQ ID No.: 27; vanYB, SEQ ID No.: 28; vanHB, SEQ ID No.: 29; vanB, SEQ ID No.: 30; vanXB, SEQ ID No.: 31; vanW, SEQ ID No.: 32 and conserved sequences thereof) SEQ ID No.: 11-12. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

iii) claims 1-5, 13-15, 24-27, 29 (partial) and 8 (complete) : the same method as invention group 1, but wherein said vancomycin resistant organism is a **vanC** resistant organism and the anti-sense molecule is selected from the group consisting of a vanC antisense molecule or vanC-2. An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the **VanC** resistance (SEQ ID No.: 3) mediated by vanC-2 gene (SEQ ID No.: 33). A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

iv) claims 1-5, 13-15, 24-27, 29 (partial) and 9 (complete) : the same method as invention group 1, but wherein said vancomycin resistant organism is a **vanD** resistant organism and the anti-sense molecule is selected from the group consisting of a vanD antisense molecule, a vanRD antisense molecule, a vanSD antisense molecule, a vanYD antisense molecule, a vanHD antisense molecule and a vanXD antisense molecule. An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the **VanD** resistance /**VanD** gene cluster of SEQ ID No.: 4 (which includes vanRD, SEQ ID No.: 34; vanSD, SEQ ID No.: 35; vanYD, SEQ ID No.: 36; vanHD, SEQ ID No.: 37; vanD, SEQ ID No.: 38; vanXD, SEQ ID No.: 39 and conserved sequences thereof) SEQ ID No.: 13. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

v) claim 20 and 22 (partial) : a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a **vanH** promoter in the organism, wherein the vanH promoter is not operatively coupled to a vancomycin resistance gene of the organism. Said method wherein the vanH promoter is contained on an enterococcus vector and enhancing expression comprises introducing into the organism an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism.

vi) claims 18, 21, 23, 28 (complete) and 20, 22 (partial) : a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a **vanH** promoter in the organism to an amount sufficient to reduce vancomycin resistance in the organism, wherein the vanH promoter is operatively coupled to an antisense vancomycin resistance molecule (or if not operatively coupled then an antisense vancomycin resistance molecule operatively coupled to a vanH promoter is

coadministered). Said method wherein the vanH promoter and the antisense vancomycin resistance molecule are contained on an enterococcus vector and enhancing expression comprises introducing into the organism an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism. A method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism a vector comprising a VanR-responsive promoter (vanH) operatively coupled to the vanA antisense molecule. A vector comprising a vanH promoter operatively coupled to an isolated nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule selected from the group consisting of SEQ ID No.: 1-13. An isolated vancomycin resistant organism comprising such a vector.

According to **Rule 13 PCT** an application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept, i.e. having at least one common technical feature defining a contribution over the known prior art. In the present case, the common technical features among the different identified groups of inventions are considered to be (**i**) the isolated nucleic acid sequences that hybridize under stringent conditions to a nucleic acid sequence conferring vancomycin resistance (in particular to SEQ ID Nos 1-13, preferably SEQ ID Nos 5-13 and more preferably SEQ ID No 5-10) (antisense sequences) and (**ii**) the use of these sequences (or parts thereof) for reducing vancomycin resistance in a vancomycin-resistant organism. However, the nucleic acid sequences responsible for vancomycin (VanA, VanB, VanC and VanD) resistance were already well known in the prior art as well as nucleic acid sequences hybridizing to these sequences and general fragments and/or portions thereof (see International Search Report, in particular WO92/07942). Thus, in view of this prior art, the first common technical feature cited above (**i**) cannot be seen as a single inventive concept anymore.

The general use of antisense antibiotic resistance molecules for inhibiting the expression of an antibiotic resistance gene and thus, reducing antibiotic resistance in an antibiotic-resistant organism was also well-known in the prior art (WO90/00624). This antisense approach had been disclosed as being useful for general antibiotic resistance and particularly referred in connection with vancomycin resistance too (see P. Mitchell, Pharmaprojects Magazine 1998,Vol. 3(8), 16-20). Moreover, (parts of) isolated nucleic acid sequences that hybridize under stringent conditions to a nucleic acid sequence conferring vancomycin resistance had already been used for inactivating (insertion by homologous

recombination) or reducing the vancomycin resistance in a vancomycin resistant organism (WO92/07942). Thus, neither the use of general antisense molecules nor the reduction of the vancomycin resistance in a vancomycin-resistant organism can be considered as a single inventive concept.

The underlying technical problem of the present invention is considered to be the provision of alternative methods for reducing vancomycin resistance and the (antisense) products therefore. Each and every group of inventions identified above provide a particular and specific solution to this technical problem. However, due to the mechanism of action and the structural differences among the different products used in each one of these above identified groups of inventions, the IPEA fails to see any common technical feature defining an inventive contribution over the known prior art and thus, the objection raised under rule 13 PCT is maintained. Furthermore, the IPEA considers that in later stages (regional phase) the above identified groups of inventions could actually be subdivided in further subgroups. The first four groups of inventions could be subdivided in isolated nucleic acids hybridizing to the different components of the corresponding Van resistances (vanA, vanR, vanS, vanH, vanRB, vanSB, etc...) and the sixth group of inventions could further be divided into the different combinations of the VanH promoter and these different components of the Van resistances.

As stated above on "Additional remarks to item III", upon invitation to pay for additional examination fees or to restrict the claimed subject matter (letter 03.07.01), the applicant with letter dated 27.07.01 has paid a further examination fee and requested the examination of the first and sixth group of inventions identified above, i.e. claims 1-5, 13-15, 24-27, 29 (partial) and claims 6, 10-12, 16-17, 19 (complete) (first group) and claims 20, 22 (partial) and claims 18, 21, 23, 28 (complete) (sixth group). Thus, the present IPER only concerns the subject matter of these claims.

5. Additional remarks to item V :

The examples of the present application disclose the production of a plasmid comprising the vanH promoter (vanHP), namely pAM401-vanHP, and a plasmid comprising both the vanH promoter and the vanA antisense, namely pAM401-vanHP-vanA antisense. There is technical data demonstrating an important decrease in the vancomycin MIC for microorganisms (Enterococcal) transformed by electroporation with these two plasmids

(16-32 μg/ml and 8 μg/ml respectively with 128 μg/ml as standard without transformation). The examples further refer to a pAMP1-vanA antisense. However, it seems to be no susceptibility data concerning said vanA antisense alone (pAMP1-vanA antisense).

The following documents have been cited in the International Search Report (ISR) as being relevant for assessing the novelty and inventiveness of the claimed subject matter:

1st invention (antisense VanA)

i) WO92/07942 (**D1**) discloses the nucleotide and the corresponding encoded amino acid sequence of VanH, VanA and VanX (and VanC). D1 refers to complementary sequences and sequences capable of hybridizing with the sequences of these disclosed Van genes (antisense DNA sequences). Moreover, D1 explicitly refers to insertional inactivation of the vancomycin resistance (resistance reduction), wherein such insertion is said to take place by homologous recombination (and thus, using such complementary and/or antisense sequences) (page 46 and page 51). Thus, this document is considered to anticipate the subject matter of claims 24-27 and 29 (Articles 33 (2) and (3) PCT).

ii) WO90/00624 (**D2**) discloses a method for treatment of bacterial diseases based on the use of antisense nucleotide sequences for reducing bacterial antibiotic resistance. D2 refers to the general use of antisense sequences for inhibiting the expression of different genes (page 4 lines 7-28) and it explicitly points out its relevance for bacterial antibiotic resistance (in particular page 4 lines 29 to page 5 lines 25). This document is, however, only exemplified by the inhibition of the E. coli macromolecular synthesis operon (MMS).

iii) this "antisense approach" is considered to be suitable and appropriate for reducing general antibiotic resistance. In fact, the document P. Mitchell, Pharmaprojects Magazine 1998, Vol. 3 (8), pages 16-20 (**D3**) explicitly refers to such an antisense approach in the general context of vancomycin resistance (pages 18-19). In this respect, document WO98/12205 (**D4**) explicitly contemplates the use of antisense sequences for inhibiting the expression of different transcriptor regulators (ivi-2, ivi-3 and ivi-4) from Enterococcus faecalis (page 3 last paragraph to page 4 first paragraph) and document WO96/08582 (**D5**) discloses the use of vanH, vanA and vanX complementary sequences (antisense) as specific probes for detection and diagnosis of antibiotic resistance genes (page 24 example 9 and page 26 example 13 as well as page 38, Table 8). Thus, D5 is considered to anticipate the subject matter of claims 24-26 too (Articles 33 (2) and (3) PCT).

This cited prior art does not anticipate the subject matter of claims 1-5, 13-15 (partial) and 6, 10-12, 16-17, 19 (complete), which is thus considered to fulfil the requirements of article 33 (2) PCT. However, the IPEA considers that the skilled person being aware of the interest of reducing the vancomycin resistance in Enterococcus (as clearly shown in documents D1, D3, etc...) and the successful use of the antisense approach for reducing antibiotic resistance (as shown in documents D2 and D3) and having at hand the specific (antisense) sequences of the VanA cluster (D1 and D5) would have had more than a reasonable expectation of success in achieving the subject matter of these claims 1-6, 10-17 and 19 without needing any special inventive contribution or skill (Article 33 (3) PCT).

6th invention (VanH promoter in combination with antisense vancomycin)

No documents have been found disclosing the specific and advantageous combination of the VanH-promoter or a VanR-responsive promoter operatively linked with a vancomycin antisense gene, which result in a double effect, namely VanR binding competition with the endogenous VanH-promoter (operatively linked to the vancomycin resistance gene cluster) and thus, lowering the level of "sense vancomycin resistance genes) and expression of antisense vancomycin resistance genes and inhibition of an expressed vancomycin resistance gene. Thus, the subject matter of claims directed to such an embodiment, i.e. claims 18, 21, 23, 28 (complete) and 20, 22 (partial) is considered to fulfil the requirements of articles 33 (2) and (3) PCT.

The attention of the Applicant is also drawn to the fact that the subject matter of examined claims 1-6 and 10-29 (complete and/or partial) can be seen as directed to methods for treatment of the human or animal body (insofar the claimed subject matter comprises methods in vivo too) and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT too. Furthermore, for such a subject matter no unified criteria exist in PCT for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

6. Additional remarks to item VIII :

The following objections are also raised under Article 6 PCT concerning the clarity of the claims :

i) the wording "anti-sense vancomycin resistance molecule" is ambiguous as far as said molecule is not clearly defined and/or characterized. It is not clear what an anti-sense molecule is intended to be (anti-sense nucleic acid ??). This objection also applies for the subject matter of other claims such as claims 6, 7, etc....

ii) the IPEA also considers that the reference to general "anti-sense nucleic acids" alone without further characterizing or defining said nucleic acid sequences, such as requiring a particular length, position in the SEQ ID No, etc... is also ambiguous. In fact, in order to achieve the desired result (i.e. being therapeutically useful so as to provide the desired resistance), the selection of very "exclusively and specifically" (anti- sense) sequences is required, namely sequences which are directed to particular (exclusive and specific) portions of the targeted vancomycin mRNA/DNA and which are able to interact with anything. The general prior art concerned with antisense methodology clearly refers to nonspecific binding and chemical and metabolic instability as well as delivery problems (see for instance Clinical Infectious Diseases).

iii) claim 2 is ambiguous. The use of "such as" is not to be seen as a limitation or a restriction to the scope of the claim. (Moreover, it is not clear whether such a wording has been omitted on purpose for the Gram-positive bacteria or not).

iv) the use of the abbreviations "VanA", "VanB", etc... must be clearly understood by the skilled person and they do not have to introduce any possible ambiguity to the claims (different possible interpretations, etc...). This objection applies to different claims such as claims 5, 6, etc.... In this respect, the references to a "complete vanA gene sequence" and/or to "conserved regions of the vanA gene sequence" without clearly indicating the complete sequence (SEQ ID No.) and said regions cannot be seen as fulfilling the requirements of Article 6 PCT (the same objection applies to claim 19). A similar objection applies to claims referring to the "active site of the ligase" (claim 12). Moreover, as far as the claims do not clearly define the "anti-sense molecule" (length, region or position, etc... see paragraph (ii) above) any (heterologous) nucleic acid can be seen as an anti-sense

Van molecule because it will surely have arbitrarily short fragments comprising parts of said molecule (one or two nucleotides).

v) In this respect, "vanR-responsive promoter" in claim 18 is not defined by any sequence (SEQ ID No). Moreover, the VanH promoter can be the VanHA, VanHB, etc... and thus, is not clear whether it is actually intended to be a generic promoter or not

vi) examples disclose the production of a plasmid comprising the vanH promoter (vanHP), namely pAM401-vanHP, and a plasmid comprising both the vanH promoter and the vanA antisense, namely pAM401-vanHP-vanA antisense, and refers to an important decrease in the vancomycin MIC (16-32 μg/ml and 8 μg/ml respectively with 128 μg/ml as standard without transformation with these plasmids). However, no demonstration of (i) vanA antisense alone (pAMP1-vanA antisense), (ii) other genetic elements of the multiple VanA operon functions (vanR, vanS, etc...) let alone (iii) from other Van operons and elements thereof (VanB operon with vanRB, vanSB, etc...; VanC, VanD operon with vanD, vanRD, etc...). No technical support seems to be found for this subject matter which thus does not fulfil the requirements of article 6 PCT in combination with Article 5 PCT.

In this respect well-known that antisense approach has specific problems such as nonspecific binding and chemical and metabolic instability (as well as major problems in delivering intact oligonucleotides to intracellular targets) (see paragraph (ii) above). Moreover, not all the vancomycin resistance genes present in a Van cluster are actually essential for the vancomycin resistance and thus, antisense oligonucleotides directed to these non-essential resistance genes would not have the desired effect.

vii) Moreover, the application demonstrates that the effect found with the vanH promoter is actually due to the presence of a pVanR binding domain within said promoter (through the binding and sequestration of pVanR from the native vanH promoter) as exemplified by plasmids comprising the vanH promoter deficient in said binding domain (pAM401-pVanR-BD-) and the effect found by the addition or transfer of said binding domain (pAM401-pVanR-BD+). Thus, reference to general pVanH promoter without requiring the complete, full-length VanH promoter or at least fragments thereof comprising the pVanR binding site is considered to be unclear (as far as it does not clearly require the presence of the "essential technical feature", namely the pVanR binding site).

viii) the general and broad wording "organism" in claim 29 could embrace human beings and thus, presenting the same ethical and moral problems associated with a claim explicitly directed to such a subject matter.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
B0662/7036WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 22086	11/08/2000	17/08/1999
Applicant		
BETH ISRAEL DIACONESS MEDICAL CENTER, INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 9 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/22086

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-23 as far as they comprise in vivo (therapeutic) methods, are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 13-15, 24-27, 29 (partial) and 6, 10-12, 16-17, 19 (complete)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism at least one anti-sense vancomycin resistance molecule under conditions to inhibit expression of a vancomycin resistance gene, wherein said vancomycin resistant organism is a vanA resistant organism and the anti-sense molecule is selected from the group consisting of a vanA antisense molecule, a vanR antisense molecule, a vanS antisense molecule, a vanH antisense molecule, a vanX antisense molecule, a vanY antisense molecule and a vanZ antisense molecule. Said method wherein the anti-sense vancomycin resistance molecule hybridizes to the complete vanA gene sequence or to a conserved region (from 10 to 30 nucleotides) thereof (encodes an active site of the ligase) or to the complete vanX gene sequence or to a conserved region thereof. Said method wherein introducing the anti-sense vancomycin resistance molecule comprises contacting the vancomycin resistant organism with at least one vector (enterococcal shuttle vector, bacteriophage, peptide nucleic acid molecule, enterococcal conjugative transposon or a pheromone-responsive plasmid) comprising one or more vanA "anti-sense vancomycin resistance molecules" under conditions to allow the vector to enter the organism and inhibit expression of one or more vancomycin resistance genes.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanA resistance/VanA gene cluster of SEQ ID No.: 1 (which includes vanR, SEQ ID No.: 18; vanS, SEQ ID No.: 19; vanH, SEQ ID No.: 20; vanA, SEQ ID No.: 21; vanX, SEQ ID No.: 22; vanY, SEQ ID No.: 23; vanZ, SEQ ID No.: 24 and conserved sequences thereof) SEQ ID No.: 5-10. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

2. Claims: 1-5, 13-15, 24-27, 29 (partial) and 7 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanB resistant organism and the anti-sense molecule is selected from the group consisting of a vanRB antisense molecule, a vanSB antisense molecule, a vanYB antisense molecule, a vanW antisense molecule, a vanHB antisense molecule and a vanXB antisense molecule.

An isolated nucleic acid that hybridizes under stringent

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

conditions to a nucleic acid molecule selected from the VanB resistance/VanB gene cluster of SEQ ID No.: 2 (which includes vanRB, SEQ ID No.: 26; vanSB, SEQ ID No.: 27; vanYB, SEQ ID No.: 28; vanHB, SEQ ID No.: 29; vanB, SEQ ID No.: 30; vanXB, SEQ ID No.: 31; vanW, SEQ ID No.: 32 and conserved sequences thereof) SEQ ID No.: 11-12. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

3. Claims: 1-5, 13-15, 24-27, 29 (partial) and 8 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanC resistant organism and the anti-sense molecule is selected from the group consisting of a vanC antisense molecule or vanC-2.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanC resistance (SEQ ID No.: 3) mediated by vanC-2 gene (SEQ ID No.: 33). A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

4. Claims: 1-5, 13-15, 24-27, 29 (partial) and 9 (complete)

Same method as invention group 1, but wherein said vancomycin resistant organism is a vanD resistant organism and the anti-sense molecule is selected from the group consisting of a vanD antisense molecule, a vanRD antisense molecule, a vanSD antisense molecule, a vanYD antisense molecule, a vanHD antisense molecule and a vanXD antisense molecule.

An isolated nucleic acid that hybridizes under stringent conditions to a nucleic acid molecule selected from the VanD resistance/VanD gene cluster of SEQ ID No.: 4 (which includes vanRD, SEQ ID No.: 34; vanSD, SEQ ID No.: 35; vanYD, SEQ ID No.: 36; vanHD, SEQ ID No.: 37; vanD, SEQ ID No.: 38; vanXD, SEQ ID No.: 39 and conserved sequences thereof) SEQ ID No.: 13. A vector comprising said isolated nucleic acid and an isolated vancomycin resistant organism comprising such a vector.

5. Claim : 20 and 22 (partial)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a vanH promoter in the organism, wherein the vanH promoter is not operatively coupled to a vancomycin resistance gene of the organism. Said method wherein the vanH promoter is contained on an enterococcus vector and enhancing expression comprises introducing into the organism

an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism.

6. Claims: 18, 21, 23, 28 (complete) and 20, 22 (partial)

a method for reducing vancomycin resistance in a vancomycin-resistant organism comprising enhancing expression of a vanH promoter in the organism to an amount sufficient to reduce vancomycin resistance in the organism, wherein the vanH promoter is operatively coupled to an antisense vancomycin resistance molecule (or if not operatively coupled then an antisense vancomycin resistance molecule operatively coupled to a vanH promoter is coadministered). Said method wherein the vanH promoter and the antisense vancomycin resistance molecule are contained on an enterococcus vector and enhancing expression comprises introducing into the organism an amount of vector to express an amount of the vanH promoter sufficient to bind to phosphorylated VanR and thereby reduce vancomycin resistance in the organism.

A method for reducing vancomycin resistance in a vancomycin-resistant organism comprising introducing into the organism a vector comprising a VanR-responsive promoter (vanH) operatively coupled to the vanA antisense molecule. A vector comprising a vanH promoter operatively coupled to an isolated nucleic acid molecule that hybridizes under strinhgent conditions to a nucleic acid molecule selected from the group consisting of SEQ ID No.: 1-13. An isolated vancomycin resistant organism comprising such a vector.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]T/US 00/22086

A. CLASSIFICATION OF SUBJECT MA[redacted]
IPC 7 C07K14/315 C12N15/11 C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 92 07942 A (PASTEUR INSTITUT) 14 May 1992 (1992-05-14) the whole document, in particular pages 7, 46 and 51	24-27,29 1-6,8, 10-17,19
Y	WO 90 00624 A (BAYLOR COLLEGE MEDICINE) 25 January 1990 (1990-01-25) the whole document, in particular page 4 line 7 to page 5 line 25	1-17,19
A	PETER MITCHELL: "Facing up to antibiotic resistance" PHARMAPROJECTS MAGAZINE, vol. 3, no. 8, June 1998 (1998-06), pages 16-20, XP000943900 the whole document, in particular pages 18-19	1-23,28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2001	18.04.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Julia, P

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 98 12205 A (VIRUS RESEARCH INST INC ;BEATTIE DAVID T (US)) 26 March 1998 (1998-03-26) page 3 last paragraph to page 4 first paragraph	1-23,28
X	WO 96 08582 A (BERGERON MICHEL G ;OUELLETTE MARC (CA); ROY PAUL H (CA)) 21 March 1996 (1996-03-21) the whole document, in particular page 17, page 24 example 9, page 26 example 13 and Table 8	24-26
P,X	DATABASE GALE GROUP NEWSLETTER DB [Online] D.J. DENOON: "Gene-Based strategy reverses vancomycin resistance" XP002154962 Database accession number 56646980 abstract & Gene Therapy Weekly 1999, Oct 18	1-6, 10-23,28
Y	STEFAN EVERS AND PATRICE COURVALIN: "Regulation of VanB-type vancomycin resistance gene expression by the VanSB-VanRB two-component regulatory system in Enterococcus faecalis V583" JOURNAL OF BACTERIOLOGY, vol. 178, no. 5, March 1996 (1996-03), pages 1302-1309, XP002153486 US the whole document	1-5,7, 13-15
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Y	the whole document, in particular pages 6 and 8-10	1-5,7, 13-15
Y	F. NAVARRO AND P. COURVALIN: "Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in Enterococcus casseliflavus and Enterococcus flavescens" ANTIMICROB AGENTS CHEMOTHER, vol. 38, no. 8, August 1994 (1994-08), pages 1788-1793, XP000984075 the whole document	1-5,8, 13-15

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Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 99 01571 A (MODRUSAN ZORA D ;ID BIOMEDICAL CORP (CA)) 14 January 1999 (1999-01-14) thw whole document, in particular claim 4 ---	24-27
X	M. ARTHUR ET AL., : "Regulated interactions between partner and non-partner sensors and response regulators that control glycopeptide resistance gene expression in enterococci" MICROBIOLOGY, vol. 145, no. PT8, August 1999 (1999-08), pages 1849-1858, XP000986365 the whole document, in particular paragraph bridging pages 1856-1857 and figure 2d ---	20,22
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Y	MOELLERING R C: "ANTIBIOTIC RESISTANCE: LESSONS FOR THE FUTURE" CLINICAL INFECTIOUS DISEASES,THE UNIVERSITY OF CHICAGO PRESS, CHICAGO, IL,US, vol. 27, no. SUPP. 01, August 1998 (1998-08), pages S135-S140, XP000943873 ISSN: 1058-4838 the whole document, in particular page S138 rigth column last paragraph and page 139 rigth column --- -/--	20,22

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International Application No
PCT/US 00/22086

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARTHUR M ET AL: "THE VANS-VANR TWO-COMPONENT REGULATORY SYSTEM CONTROLS SYNTHESIS OF DEPSIPEPTIDE PEPTIDOGLYCAN PRECURSORS IN ENTEROCOCCUS FAECIUM BM4147" JOURNAL OF BACTERIOLOGY,WASHINGTON, DC,US, vol. 174, no. 8, April 1992 (1992-04), pages 2582-2591, XP000944110 ISSN: 0021-9193 cited in the application the whole document, in particular page 2587 left column and page 2588 left column second full-paragraph -----	20,22

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Information on patent family members

International Application No
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